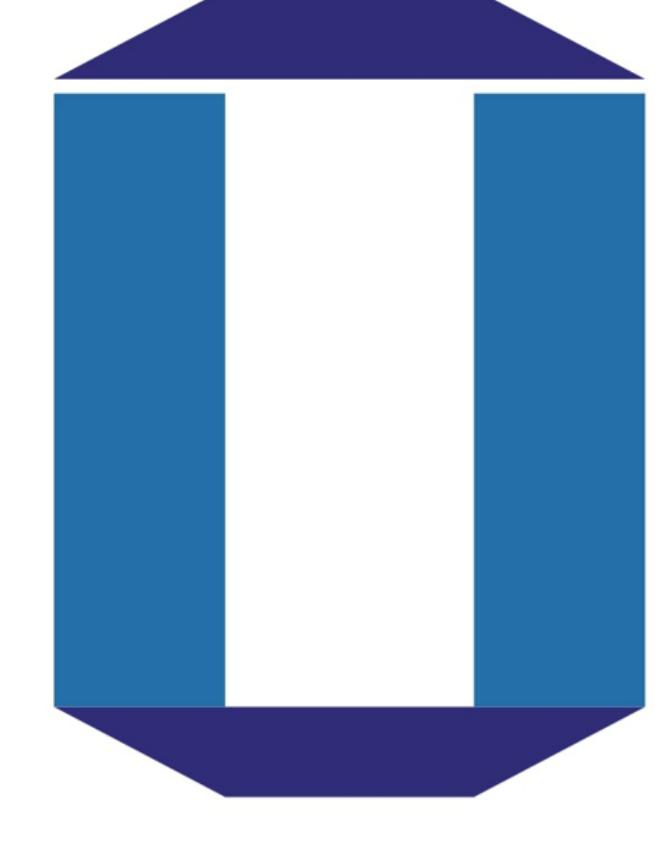


# Mechanical signals influence circadian clock genes in murine osteocytes: possible role in the orthodontic tooth movement



発表内容に沿って演題名を一部修正しております

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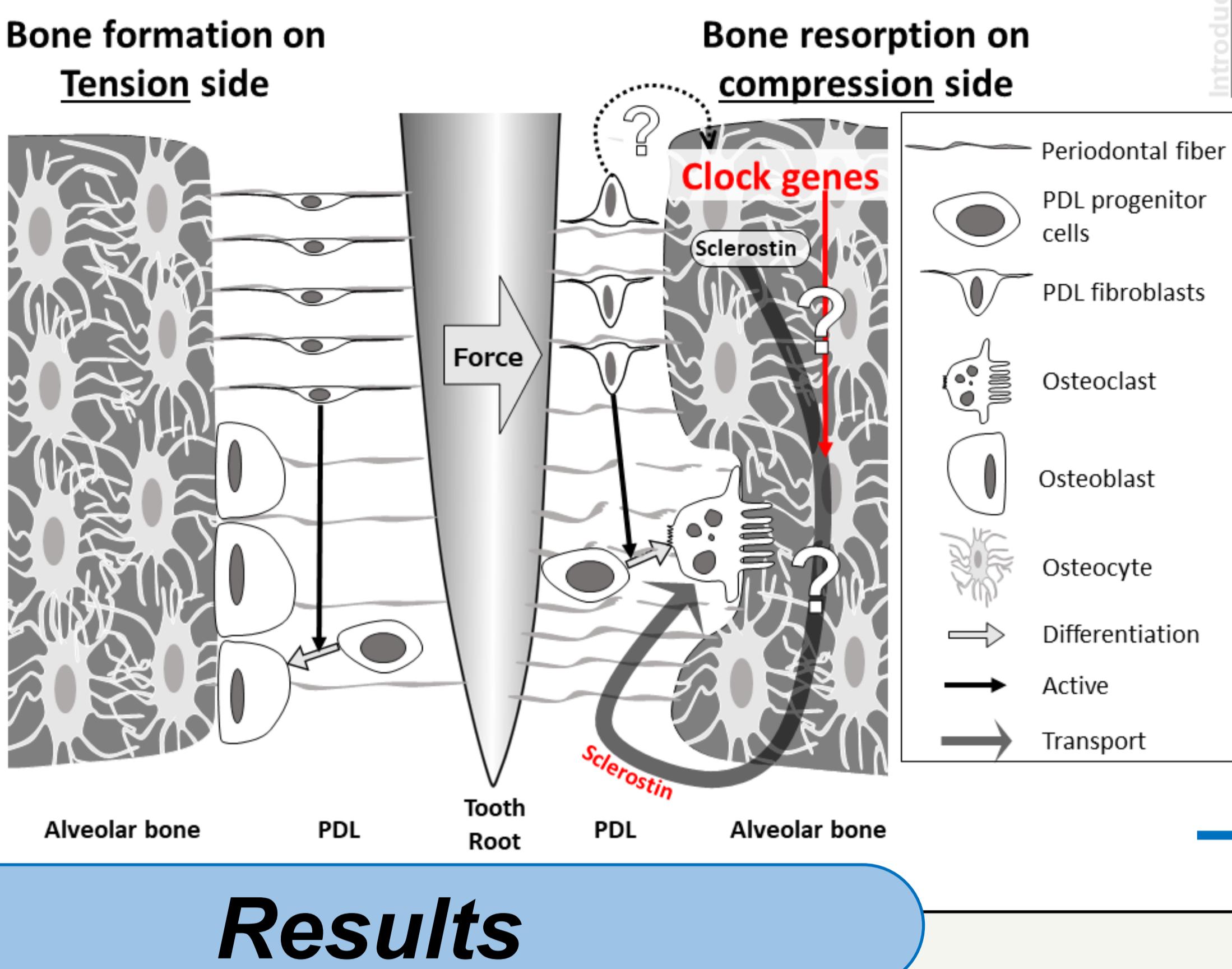
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## Introduction

The present work was supported by Grant-in-Aid for Scientific Research (to Y. Ishihara [17H04413] and H. Kamioka [16H05549]) from the Japan Society for the Promotion of Science, Japan.

It is well established that osteocytes and their dendritic process network are the main components involved in mechanosensing and mechanotransduction. Sclerostin is a key determinant of the bone mass which mainly produced by osteocytes. The purpose of this study was to investigate the mechano-induced spatiotemporal regulation of sclerostin in alveolar osteocytes generated in response to experimental tooth movement in mice. We also employed an integrative bioinformatics approach to explore potential mechanisms underlying the regulation of sclerostin by bone remodeling and provided a possible association with biological processes related to the peripheral circadian rhythm. Finally, an in vitro co-culture model was applied for the preliminarily discussion of the role of periodontal ligament (PDL) cells-osteocytes crosstalk in the mechanotransduction during **orthodontic tooth movement (OTM)**.



## Material & Methods

Number of sclerostin positive cells

Sclerostin profile

Mean

COV

Hig5

Low5

MPF

To quantify the spatial distribution of sclerostin

Statistical correlation between 1 and 2

Bioinformatic data digging and validation & Numerical simulation

Mouse model and analysis method

A) System of orthodontic tooth movement in mouse  
B) Histogram analysis method (COV, coefficient of variation).

C) A real example showed the setting of ROI and reference part, generation of profile and histogram and calibration of histogram.

D) Calibration of fluorescence intensity profile of sclerostin.

E) Power spectral density was generated by fast Fourier transform and the calculation of mean power spectral density frequency (MPF)

**The immunostaining results (1&2)**

A) The nuclei were stained with DAPI (blue) and the sclerostin were stain in green. Arrows indicate the orthodontic force direction

B) The double-labeling fluorescence image shows the sclerostin positive osteocytes in alveolar bone.

C) Sclerostin profile on compression side, tension side and reference part.

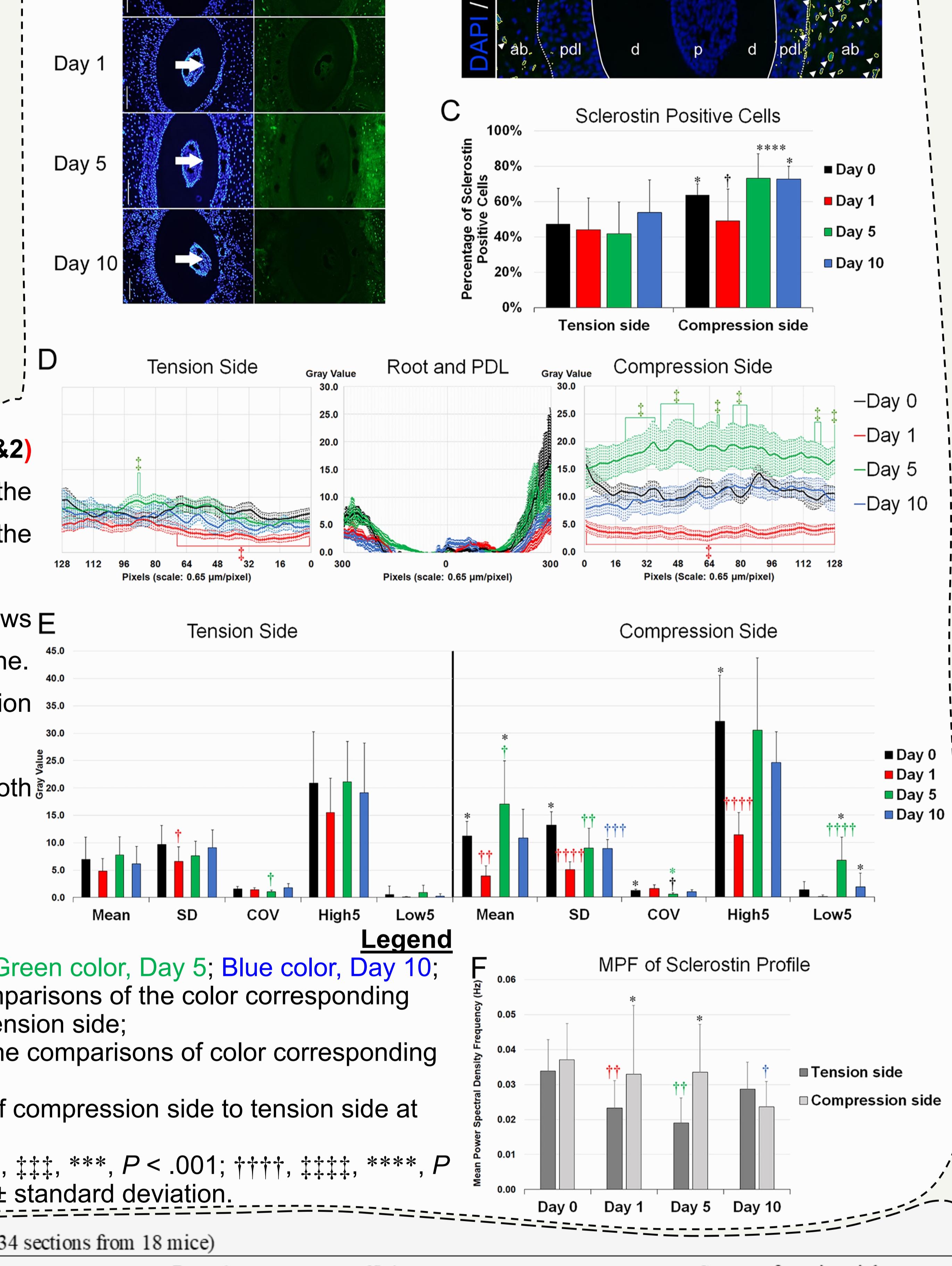
D) Histogram analysis of sclerostin on both compression and tension side.

E) The mean power frequency (MPF) results.

F) The percentage of sclerostin positive cells.

## Results

1. Expression change of sclerostin in alveolar osteocytes during OTM
2. Changes in spatial distribution of sclerostin in alveolar bone during OTM
3. Spatial distribution of sclerostin has biological meaning
4. Clock genes were possible to regulate the spatial distribution of sclerostin



Dark color, Day 0; Red color, Day 1; Green color, Day 5; Blue color, Day 10;  
†, unpaired t-test for the multiple comparisons of the color corresponding Day to Day 0 on compression or tension side;  
‡, Fisher's LSD t-test in ANOVA for the comparisons of color corresponding Day to Day 0;  
\*, paired t-test for the comparisons of compression side to tension side at each day.  
†, ‡, \*, P < .05. ††, ‡‡, \*\*, P < .01. †††, ‡‡‡, \*\*\*, P < .001. ††††, ‡‡‡‡, \*\*\*\*\*, P < .0001. All the values are the mean ± standard deviation.

Table 2. The results of multiple linear regression <sup>a</sup> (34 sections from 18 mice)							
R <sup>2</sup>	F statistic	P - Value	Coefficient estimate ± SE	t value	P - Value	Square of semipartial correlations	VIF <sup>b</sup>
The percentage of sclerostin positive cells	0.38	13.067	0.00000093				
Intercept			36.12 ± 5.62	6.43	0.00000002		
The MPF of sclerostin profile			373.25 ± 153.92	2.43	0.01814740	0.06	1.06
Mean			1.61 ± 0.31	5.27	0.00000171	0.27	1.22
Low5			-1.66 ± 0.34	-3.43	0.00105715	0.11	1.29

VIF, variance inflation factor; b: VIF higher than 10 suggests a linear relationship between the predictors; SE: standard error.

a: Mixed compression, tension side and every time point; \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001

**Correlation analysis (3)** Table 1. Significant related factors<sup>a</sup> (34 sections from 18 mice)

Spearman's rank test and multiple linear regression analysis indicated the significant correlation between the number of sclerostin positive cells and the spatial distribution of sclerostin in alveolar bone during OTM.	
MPF of sclerostin profile	0.354

Factors relative to the percentage of sclerostin positive cells	
MPF of sclerostin profile	0.354
Mean	0.428
SD	0.363
COV	-0.243
High5	0.482

Spearman's correlation coefficient P - Value

Mixed compression, tension side and every time point. MPF: mean power frequency.

**Protein-protein interactions network**

Legend

Number of nodes: 24 Number of edges: 35 PPI enrichment p-value: <1.0 × 10<sup>-16</sup>

Interaction confidence was represented as the color of edge stroke

Topological property was represented as the color of node fill

KEGG enrichment results

Accession Number Annotation log(1/FDR) 0 5 10 15 20

mmu04060 Cytokine-cytokine receptor interaction

mmu04661 TNF signaling pathway

mmu04350 TGF-beta signaling pathway

mmu04064 NF-kappa B signaling pathway

mmu04062 Chemokine signaling pathway

Legend

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